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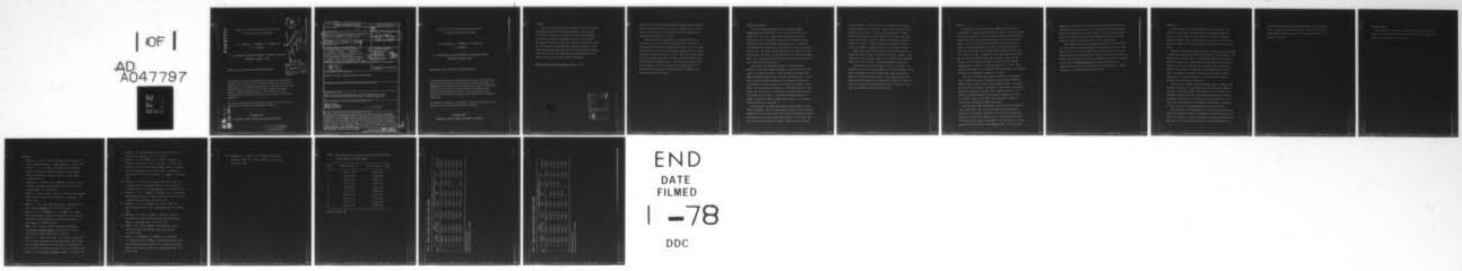

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Recovery of alveolar macrophages from rhesus and cynomolgus monkeys by lung lavage

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Running head: LUNG LAVAGE AND ALVEOLAR MACROPHAGES

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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A lung lavage technique has been developed to recover alveolar macrophages from rhesus and cynomolgus monkeys. Sterile saline was injected through an endotracheal tube in anesthetized monkeys and then lung wash fluids containing leukocytes were withdrawn. The lung wash fluids from each monkey routinely contained more than 20×10^6 leukocytes of which 80% were alveolar macrophages. Lung lavage has been performed weekly for 6 wk in both species of monkey with no ill effects. This technique has many applications in the study of infection and pulmonary defense mechanisms.

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ABSTRACT

A lung lavage technique has been developed to recover alveolar macrophages from rhesus and cynomolgus monkeys. Sterile saline was injected through an endotracheal tube in anesthetized monkeys and then lung wash fluids containing leukocytes were withdrawn. The lung wash fluids from each monkey routinely contained more than 20×10^6 leukocytes of which 80% were alveolar macrophages. Lung lavage has been performed weekly for 6 wk in both species of monkey with no ill effects. This technique has many applications in the study of infection and pulmonary defense mechanisms.

Macaca mulatta; Macaca fasicularis; phagocytic cells

LAVAGE IS AN EFFECTIVE MEANS OF REMOVING PARTICULATE MATTER FROM THE lungs. This technique has been assessed in animals (8, 12, 13) and has been extensively used in the treatment of human patients with obstructive lung diseases (14, 16-18).

Pulmonary alveolar macrophages are relatively difficult to obtain and, in the past, studies requiring harvest of these cells have necessitated killing the experimental animal (1, 10, 11). Lung lavage has been used to obtain alveolar macrophages from living small laboratory animals (9) and man (2, 6, 15). The present study reports the use of repeated lung lavage to obtain alveolar macrophages from live rhesus and cynomolgus monkeys. The alveolar macrophage is the primary phagocytic cell of the lung (3-5), and knowledge of its activities may be essential in understanding the pathogenesis and therapy of respiratory infection.

METHODS AND MATERIALS

Six rhesus (Macaca mulatta) and five cynomolgus (Macaca fasicularis) monkeys, weighing 5.4 to 10.5 kg and 3.9 to 4.8 kg, respectively, were used. Monkeys were chemically restrained by intramuscular (i.m.) injection of ketamine HCl (10 mg/kg) followed by atropine sulfate (0.04 mg/kg) subcutaneously (s.c.). Halothane in 100% oxygen was administered by face mask until surgical anesthesia was achieved. The pharynx, larynx and trachea were anesthetized with 2% lidocaine spray and the trachea intubated with a cuffed endotracheal tube. The cuff was inflated and subsequent ventilation was maintained by intermittent positive pressure.

Lung lavage was performed according to the method used by Mauderly (9) with small laboratory rodents, except that only the left lung of each monkey was washed. After anesthesia was induced, each monkey was hyperventilated until it became temporarily apneic. During apnea, a syringe-manometer system was used to measure the volume of air required to inflate the lungs to a transthoracic pressure of 20 cm water. For the purpose of calculation, it was assumed that the volume of the left lung is equal to one-half the total lung volume measured at a pressure of 20 cm water. To provide an additional margin of safety, the maximum volume of a single wash was 80% of the calculated volume and never more than 50 ml.

Lung lavage was performed during apnea with the monkeys in left lateral recumbency. The calculated volume of 0.9% sterile NaCl (saline) warmed to 37°C was slowly injected from a sterile 50-ml syringe through the endotracheal tube and carried by gravity into the left lung. The wash fluid was then collected by gentle withdrawal into the syringe.

A water manometer to monitor pressure was attached to the syringe and endotracheal tube by a T-tube. Pressure never exceeded +10 cm H_2O during injection nor -10 cm H_2O during withdrawal. Repeated injection and withdrawal, using a fresh volume of saline each time, constituted a lavage. The first study used 10 volumes of saline to determine the number of washes required for sufficient recovery of alveolar macrophages; subsequent studies used 6 volumes of saline.

Monkeys were again hyperventilated to apnea after withdrawal of every third wash or whenever spontaneous respiration returned. After withdrawal of the last wash, monkeys were ventilated twice with 100% oxygen and allowed to recover from anesthesia before extubation.

Lung wash fluids were centrifuged at 230 x g for 10 min. The supernatant was decanted and the packed cells were resuspended in 10 ml Earle's 199 medium containing 10% homologous serum, 100 units/ml penicillin and 100 µg/ml streptomycin. Total leukocytes from lung wash and peripheral blood were enumerated and differential counts were performed on smears stained with Wright's stain. Viability of lung cells was determined by trypan blue exclusion.

RESULTS

To determine the efficiency of the technique for the recovery of leukocytes, lung lavage was performed on a group of six rhesus monkeys using ten 50-ml volumes of saline. The mean volume of fluid recovered and the mean number of leukocytes contained in each wash is shown in Table 1. Fluid recovery was usually least from the first wash, but generally increased with subsequent washes. The amount of saline remaining in the lungs after ten 50-ml washes ranged from 28 to 137 ml with a mean of 61.3 ml. In general, the smallest number of leukocytes was recovered from the first wash and the greatest number from the second. The total number of leukocytes recovered after 10 washes ranged between 7.45 and 54.7×10^6 with a mean of 22.7×10^6. Fluid recovered from washes 1 to 6 contained 77% of the leukocytes and therefore subsequent lung lavages were limited to six washes.

The effect of repeated lung lavage on the health of the monkey and the numbers and types of leukocytes recovered was determined by weekly lavage of groups of rhesus and cynomolgus monkeys. The results are shown in Tables 2 and 3, respectively. At least 80% of the saline used in the lavage was recovered. Total leukocyte recovery varied greatly in individual monkeys of each species, ranging from a low of 3.1×10^6 to a high of 2.98×10^7. The mean number of leukocytes recovered was unaffected by weekly lung lavage.

The major difference between the two species was the differential count of lung cells. The alveolar macrophage was the predominant leukocyte in both groups, ranging from 59 to 74% in the rhesus group and from 87 to 94% in the cynomolgus group. This difference was due to the greater percentage of eosinophils in the rhesus group, 15 to 34% compared with 0.5 to 2.4% in the cynomolgus group. The percentage of

lymphocytes, polymorphonuclear leukocytes and basophils was similar in both groups as was the cell viability. Total and differential leukocyte counts in peripheral blood were not different from normal monkey colony values and no significant changes were observed during the 6-wk study. Eosinophil counts in blood ranged from 2 to 5% in each group.

Three monkeys died during the experimental period. One rhesus and one cynomolgus monkey died during induction of anesthesia prior to lung lavage in the fifth week and another rhesus died during the sixth week. Histopathological findings indicated no gross or microscopic differences in washed lungs and lungs which had not been washed. Except for the presence of mite lesions (Pneumonyssus simicola) in the rhesus monkeys, no significant pathology was present in lungs from either species. Monkeys surviving the study remained apparently healthy.

DISCUSSION

We have developed a technique whereby repeated lung washes with saline can be performed on living rhesus and cynomolgus monkeys with recovery of large numbers of viable alveolar macrophages. Repeated weekly lung lavages produce no respiratory infection, lung lesions, nor significant changes in total and differential peripheral blood leukocyte counts.

The number of leukocytes removed from lung lavages varied considerably in individual monkeys, but no trends were observed in either species. This indicates that repeated removal of cells neither depleted leukocytes nor stimulated leukocytosis. Higher numbers of eosinophils were recovered from the lungs of the rhesus than the cynomolgus group, but there was no difference in the number of eosinophils in peripheral blood of either group. Although more eosinophils recovered from the lungs of rhesus monkeys suggest a species difference, it is also possible that this difference is attributable to parasitism.

The lung mite is common in the rhesus monkey and less common in the cynomolgus monkey (7). In this study, lung mite lesions were present in the lungs of both rhesus monkeys that died but not in the lungs of the cynomolgus. While these lesions are frequently infiltrated by numerous eosinophils, and therefore are a possible source of these cells in the rhesus lung, data presented in this study are insufficient to determine if this is the reason for the difference between the two species.

The capability of repeated sampling of alveolar macrophages without harm to the monkey offers distinct advantages in experimental design and obvious cost benefits. In our investigations, sequential sampling permits measurement of cellular metabolism, enzyme levels, and phagocytic and

bactericidal activities of alveolar macrophages before, during and after experimental respiratory infection. These studies provide basic knowledge about the role of the alveolar macrophage in the defense of the lung.

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TABLE 1. Fluid and leukocyte recovery from each 50 ml of saline used in lung lavage of 6 rhesus monkeys

Wash	Volume Recovered, ml	No. of Leukocytes ($x10^6$)
1	21.7 ± 3.9	0.65 ± 0.79
2	41.5 ± 13.9	6.10 ± 9.46
3	41.8 ± 4.2	3.10 ± 2.65
4	37.7 ± 10.7	3.26 ± 4.11
5	45.2 ± 10.7	2.48 ± 1.34
6	45.5 ± 6.7	2.08 ± 0.44
7	49.2 ± 5.7	1.86 ± 0.48
8	46.7 ± 7.2	1.41 ± 0.50
9	47.7 ± 7.6	1.27 ± 0.23
10	44.5 ± 5.0	0.82 ± 0.26

Values are means ± SD.

TABLE 2. Results of weekly lung lavage of rhesus monkeys

Week	No.	% fluid recovery	Total leukocyte count ($x10^6$)	Differential leukocyte counts					% Viability
				% Macro	% Lymph	% PMN	% BAS	% EO	
1	6	80±10.8	26.9±39.7	73.7±9.9	10.0±5.4	1.0±0.6	0.5±0.8	15.0±13.0	76.0±3.4
2	6	88±3.0	49.1±27.5	64.0±22.0	10.0±3.8	0.5±0.8	0.7±0.8	25.0±22.0	81.0±5.1
3	6	84±7.3	30.2±19.0	63.0±14.0	13.0±8.9	2.0±4.0	0.3±0.5	22.0±19.0	88.0±7.0
4	6	87±3.9	41.2±31.1	66.0±14.0	11.0±4.9	0.2±0.4	0	23.0±18.0	93.0±2.2
5	5	85±8.8	34.8±19.2	59.0±9.7	7.4±2.7	0.2±0.5	0	34.0±10.0	95.0±2.5
6	4	83±8.2	21.3±14.3	70.0±9.8	13.0±9.8	0.3±0.5	0.3±0.5	15.0±8.4	95.0±1.7

Values are means ± SD.

Each lavage consisted of 6 washes.

TABLE 3. Results of weekly lung lavage of cynomolgus monkeys

Week	No.	% fluid recovery	Total leukocyte count ($x10^6$)	Differential leukocyte counts					% Viability
				% Macro	% Lymph	% PMN	% BAS	% EO	
1	5	87±2.3	19.4±5.6	90.0±5.3	8.6±2.9	0	0.2±0.5	1.6±2.6	94.0±3.2
2	5	87±3.8	21.0±5.3	89.6±6.7	6.6±3.1	0.4±0.6	0.4±0.6	3.6±3.4	91.0±4.7
3	5	88±4.2	155.6±83.4	87.0±3.9	9.8±5.2	0.6±0.9	0.2±0.5	2.0±1.7	92.0±3.1
4	5	88±2.9	67.2±113.4	89.0±3.6	7.8±2.8	0.2±0.5	0.2±0.5	2.4±1.7	93.0±3.0
5	4	89±0.9	19.3±5.1	91.0±1.3	6.0±3.0	0.5±1.0	0	2.3±2.0	94.0±2.7
6	4	86±1.2	19.0±11.7	94.0±3.2	5.5±2.7	0	0	0.5±0.6	94.0±2.1

Values are means ± SD.

Each lavage consists of 6 washes.